Vol. XV. *August, 1908.* *No. 3.*

BIOLOGICAL BULLETIN

THE SEGREGATION OF THE GERM-CELLS OF PHRYNOSOMA CORNUTUM: PRELIMINARY NOTE.[1]

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The changes that have been effected in the theories of germ-cell origin since 1870 are characteristic of the advance of scientific thought in general. Waldeyer's theory (1870) of their origin from peritoneal cells was, in its very simplicity, so attractive that it received universal recognition; and since Nussbaum's opposing theory (1880) that the germ-cells are to be observed in the late cleavage stages of the egg, there has been continual controversy over every new detail. The phenomenon of migration of the germ-cells was observed in sponges long before Nussbaum; Balfour's evidence (1878) indicates a migration from the mesentery to the genital ridges, and Balfour admits the possibility of a migration from the blastoderm; Weismann proved conclusively its occurrence in the hydroid polyps; and yet modern embryologists seem slow to admit that the primitive germ-cells in vertebrates have the power of independent movement.

Eigenmann (1891) was the first to give a detailed account of the wanderings of the germ-cells in vertebrates; he described the migration from before backward in *Cymatogaster* (*Micrometrus*), and in 1896, a dorso-median migration in this teleost. Hoffmann (1892) noted a probable migration from the connective tissue between splanchnic mesoblast and entoblast to the germinal epithelium, and Rabl (1896) gave a detailed description of their very general distribution in selachians. It would seem that

[1] Contributions from the Zoölogical Laboratory of The University of Texas, No. 93.

Wheeler's (1899) work on the lamprey is conclusive evidence; he found the germ-cells first in the entoblast lateral to the myotome, in the mesoblast after it is cut off, and then moving into the median line. Bouin (1900) argues that the germ-cells arise directly from the peritoneal and sclerotome cells of the germinal region, but admits that they may also come from the yolk-sac. Nussbaum (1901) records their migration in *Gallus* from the splanchnic mesoblast, where they appear as large, yolk-laden cells, the ova being their descendants. Woods (1902) gives an interesting account of the germ-cells in *Acanthias*, where they first appear in the entoblast or in the yolk, migrating to the germ-gland anlagen.

Beard (1902) studied the germ-cells in various selachians. In *Raja* he traced them back to late cleavage stages; many come to lie in the germinal cavity, whence they migrate into the embryo, and along the space between entoblast and mesoblast to the germinal nidus; some leave this normal path and reach other organs of the embryo. He considers that some of these latter degenerate.

Böhi (1904) found that the first germ-cells appear in the trout-embryo not before the twenty-fifth day after fertilization; in the salmon, not before the thirty-first. They lie in the splanchnic and somatic mesoblast, being pushed into the somatic by the growth of the splanchnic.

Allen (1906) working on *Chrysemys*, found that the germ-cells originate in the entoblast at the edge of the area pellucida, in a zone extending from a point opposite the anterior end of the pronephros to a point behind the embryo. They migrate in the entoblast to a point beneath the notochord, and upward through the sclerotome to the germ-gland anlagen. A part of these also "lose the way." In *Rana pipiens* (1907) he finds that they develop from a group of apparently indifferent entoblast cells in the dorso-median region; this group is cut off from the other entoblast cells by the growth of the mesentery, or more probably, moves above the mesentery; later, the cells migrate laterally coming to lie in the paired germ-gland anlagen.

Through the courtesy of Dr. Allen, I have been enabled to examine the more important stages in the migration of the germ-cells of *Chrysemys;* they are similar to my own material, as my

conclusions, although differing from Dr. Allen's in details of early distribution and period of migration, uphold his.

My work was done on *Phrynosoma cornutum* Harlan, of central Texas. The material was largely supplied from the collections of Miss Augusta Rucker; I take this opportunity to express my thanks to her, and also to Dr. Thomas H. Montgomery, under whose direction this study was completed.

The embryos were fixed in nitric acid, Zenker's fluid, and picro-sulphuric acid, the last proving to be the most satisfactory; the sections were double stained with Heidenhain's iron hæmatoxylin and alcoholic eosin, and sections were made 6 micra thick.

The most careful study was given to series No. 26; a stage where the intestine is completely closed except at the region of the yolk-stalk which is 1.5 mm. wide; the allantois is well developed, and two visceral clefts have broken through. This was compared with series No. 12, an embryo with five visceral clefts. Each section was examined under an immersion lens, and the number and positions of the germ-cells were tabulated. In series No. 12, the peritoneum of the germ-gland anlagen is distinctly thickened throughout the region of the mesonephros; within these thickenings there are found at intervals large rounded cells, with clear cytoplasm containing yolk and large clear nuclei with deep-staining chromatin granules. Two of these are drawn in Fig. 1; the nucleus of the left germ-cell measures 6 micra and 5 micra in its diameters; that of the right, 7 micra and 6 micra. These were called typical germ-cells; considering their appearance and position, no other satisfactory explanation of their nature

	Series 12.	Series 26.	Series 27.
Sclerotome: lateral to central nervous system		4	one row of sec.
Sclerotome: lateral to aorta	9	1	
Sclerotome: within mesentery	22	5	7
Sclerotome: of yolk-stalk	4		2
Mesothelium	3	4	
Germinal anlage	44	2	2
Ectoblast		1	
Entoblast: of axial intestine	8		
Entoblast: of yolk-stalk	6		6
Cavity of yolk-stalk	2		
Total	98	17	17

can be given. Their characters were taken as criteria in studying the germ-cells occurring elsewhere.

Similar cells are observed in various regions of the embryo. The above table shows their distribution in series 12 and 26 and one row of sections of series 27, an embryo with three vis-

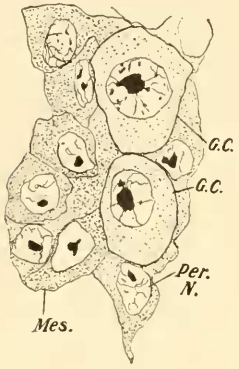

FIG. 1.

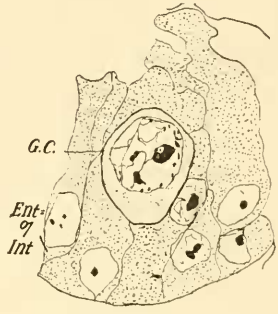

FIG. 2.

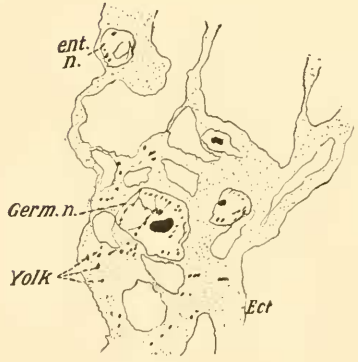

FIG. 3.

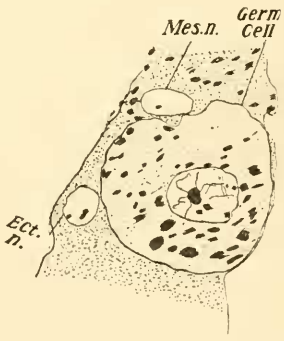

FIG. 4.

ceral clefts, only the row of sections cutting the yolk-stalk being studied.

The germ-cells in the intestinal wall (see Fig. 2), are near the region of the yolk-stalk, a point to be noticed in considering the probable path of migration.

The germ-cells in No. 26 near the central nervous system are interesting, from their location in the anterior portion of the

embryo; one is lateral to the prosencephalon, one to the diencephalon, and one to the mesencephalon, the fourth being in the region of the spinal cord. Also worthy of note are the two within the cavity of the yolk-stalk in series 12, and a third, in the section preceding, which lies partly in the entoblast, partly in the cavity of the yolk-stalk, into which it is apparently migrating.

The figures in series 12 have only comparative, not absolute, value, owing to the facts that some sections were folded so that it was impossible to determine whether or not they contained germ-cells, and that I counted only those cells the nature of which is indisputable. An exact count would undoubtedly give a larger total than 98; but this, when compared with the total of 17 in the younger stage No. 26, requires some explanation of the increase in number.

Mitoses, although frequent among the somatic cells, were not observed among the germ-cells, indicating the period of rest for the germ-cells observed in other vertebrates, and excluding the explanation of the increase in number of germ-cells by division. Of the three other possible explanations, namely the transformation of somatic cells, or of undifferentiated cells within the embryo, into germ-cells, and the migration of germ-cells from the extra-embryonic region into the embryo, the last seems to be the correct one; because, first, no "transitional cells" were observed, and, second, because germ-cells were found in the blastoderm, and in various positions indicating a migration thence into the embryo.

Practically every section of the area vasculosa in series No. 26 contains germ-cells in the entoblast, cephalad, caudad, and laterad of the embryo. I have not counted them in the entire series, but there are several in each section examined, and must be very numerous. These cells have all the characteristics of germ-cells, the nuclei of the surrounding entoblast, mesoblast, and ectoblast cells being distinctly smaller, as shown in Figs. 3 and 4. The drawings do not show so clearly as I could wish that the germ-cells lie in the entoblast rather than in the mesoblast; in fact, their size makes the question difficult to determine, especially since entoblast and mesoblast are not very clearly differentiated; but the nuclei, and often the entire cells, lie below the level of

the blood-vessels, and the lower wall of the cells, where distinguishable, touches the boundary of the entoblast, so that I conclude that they lie normally in the entoblast.

No germ-cells were observed in the extra-embryonic blastoderm of No. 12; in series No. 15, an embryo of the same age, of which about one half the sections were examined in detail, one germ-cell was found in the blastoderm; they were present in the blastoderm of series No. 27.

The path of normal migration is the entoblast of blastoderm, yolk-stalk, and intestine, and the sclerotome of the mesentery to the germinal anlagen; the only other possible path is the mesoblast of the same structures, and the following table shows that the first is the normal path:

	Series 12.	Series 26	Series 27.
Ento. of blastoderm	0	numerous	numerous
Ento. of yolk-stalk	6		6
Ento. of intestine	8		2
Scler. between intestine and germ anlage.	22	9	7
Total in path of normal migration	36	9	15
In meso. of yolk-stalk	4	0	2

Those cells that "lose the way" are found in the various positions tabulated above. It will be observed that the migration is just beginning in series 26, and practically completed in No. 12.

This controverts the suggestion that the cells are passively carried by the concrescence of the germ-layers, since this growth is too slight, between the stages studied, to account for the transposition of the germ-cells.

Now the total number of germ-cells in the older embryo, even allowing for the folded sections, is not nearly equal to the total number of extra-embryonic germ-cells in the younger embryo. The most obvious explanation of the deficit is Beard's theory of degeneration. My observations appear to uphold Beard; I find cells resembling germ-cells in every respect except the size of the nuclei, these being about equal to the somatic nuclei, or apparently absent entirely. They are especially numerous in the sclerotome around the aorta, where the abnormally placed cells are also most numerous. They occur in the cavity of the yolk-stalk, and apparently in the intestinal lumen; the presence of

two indubitable germ-cells in the cavity of the yolk-stalk, and of a third half imbedded in the entoblast and half projecting into the same cavity, lends further support to the theory. However, it will require an exact enumeration of the blastodermic and embryonic germ-cells of many embryos at various stages, as well as work along other lines, to determine the question in *Phrynosoma*. The possibility that these might be transitional forms between somatic and germ-cells is precluded, it seems to me, by the fact that they are not found in what I have termed the path of normal migration of the germ-cells, and especially by their non-appearance in the germinal anlagen.

Summary.

1. The germ-cells appear first in the entoblast of the vascular area of the blastoderm. They lie cephalad, caudad, and laterad to the embryo.

2. The germ-cells migrate in the entoblast of the blastoderm, yolk-stalk, and intestine, and the sclerotome of the mesentery, to the germinal anlagen. Very many leave this path and come to lie in various regions of the embryo.

3. Some of these abnormally placed germ-cells probably degenerate.

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